1.0. ONLINE SUPPLEMENTARY FILE

Quantitative PCR (GeneXpert® MTB/RIF) for the diagnosis of tuberculous meningitis in a high burden setting: a prospective study

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Methods

Written consent was obtained from the patient or a close relative. If patients were unable to give consent and a lumbar puncture (LP) was clinically indicated the Head of Department was approached for consent (7). Patients were clinically assessed, had a computerised tomography (CT) scan done to exclude contraindications to a lumbar puncture, and blood drawn for routine tests including serum fluorescent treponemal antibody (FTA), venereal disease research laboratory test (VDRL), HIV enzyme linked immunosorbent assay and CD4 counts. Approximately 15ml of CSF, obtained by lumbar puncture, was processed for the following tests: microscopy (Gram stain and fluorescent staining for acid-fast bacilli [auramine]), bacterial culture, *Mycobacterium tuberculosis (M.tb.)* culture (Bactec 960 MGIT; Becton Dickinson Diagnostic Systems Sparks, MD), fungal culture, cryptococcal latex agglutination test Amplicor® PCR Mycobacterium Tuberculosis Test (Roche® Diagnostic Systems Inc., Branchburg, New Jersey) (Amplicor®PCR), routine chemistry (protein, glucose, chloride), viral PCR for cytomegalovirus, varicella zoster virus, herpes simplex, VDRL, FTA and cysticercus antibodies. An uncentrifuged specimen, sample of CSF was biobanked for Xpert®MTB/RIF analysis. The clinical information recorded included demographic information, duration of symptoms and whether patients were already on anti-tuberculous or steroid therapy, HIV status, past history of tuberculosis and history of TB contact.

Categorisation of patients

Patients were categorised, based on standardised published diagnostic criteria, as definite TBM if the CSF *M.tb.* culture and/or Amplicor®PCR was positive(3, 10), probable TBM (treated empirically with anti-TB drugs but not meeting the definite TBM criteria) or non-

TBM (alternate diagnosis confirmed and response to therapy documented in the absence of anti-TB treatment) (9).

Amplicor

One hundred and forty eight were processed by an independent laboratory using the Amplicor kit, for the detection of *M.tb.* This was done as per manufacturer's protocol. Briefly, 0.5 ml of CSF was used to extract DNA using the Roche Magnapure automated DNA extraction system using the DNA 1 High performance kit. Extracted DNA was then amplified using the biotinylated primers KY18 and KY75 as described in the kit protocol. (Roche Amplicor mycobacterium tuberculosis test). PCR products were detected by the Cobas Amplicor analyser according to the kit protocol.

GeneXpert® assay and related bacterial load studies

The Xpert® MTB/RIF (Cepheid, Sunnyvale, CA) is an integrated automated sample processing and real-time polymerase chain reaction (RT-PCR) platform developed to simultaneously detect *Mycobacterium tuberculosis* (*M.tb.*) and rifampicin (RIF) resistance in a single-use cartridge hands-free step (4-6). The Xpert® MTB/RIF assay consists of two main components, namely, a Xpert® MTB/RIF plastic cartridge (containing the liquid sample processing and PCR buffers, and lyophilized real-time PCR reagents with internal sample processing and PCR probe quality controls), and the automated Xpert® MTB/RIF machine (which controls the advanced automated portion of the procedure involving the engagement of the fluidics system within the cartridge, ultrasound lysis and the performance of the real-time PCR analysis) (1, 2).

Previously stored (-70^0) , uncentrifuged samples (n = 148) were processed at the Lung Infection and Immunity Unit Laboratory (Department of Medicine, Groote Schuur Hospital, University of Cape Town) for Xpert® MTB/RIF analysis. The laboratory technician performing the Xpert® MTB/RIF assay was blinded to all subject details.

Samples were prepared according to the manufacturer's instructions (1). Briefly, the frozen, unprocessed samples were thawed and immediately processed. The CSF/SR mixture was incubated at room temperature for a total of fifteen minutes with a second shake occurring at 10 minutes. Two millilitres of the digested mixture was then transferred to the Xpert® MTB/RIF cartridge. The automated steps of the procedure were initiated by placing the loaded assay cartridge into the Xpert® MTB/RIF instrument module and then selecting the "*M. tuberculosis*-Rif" automated detection test option from the included software. The test was started within 30 minutes of adding the sample to the cartridge. Results were interpreted using the automated software. The data analysis algorithms identify the following: 1) '*M.tb* detected' if the *M.tb.* target DNA (*rpoB*) region was detected, and 2) '*M.tb.* not detected' if the *M.tb.* target DNA (*rpoB*) region was not detected. If *M.tb.* was detected the results were further categorised into 'RIF-resistance detected' (if a mutation in the *rpoB* gene was detected) and 'RIF-resistance not detected' (if no mutation was detected in the *rpoB* region). The detailed principle of the procedure, steps of the automated assay protocol and full details of the diagnostic algorithms and threshold are described in the manufacturer's package insert.

Preliminary experiments were performed to determine the detection limit for Xpert® MTB/RIF. Patients with motor neuron disease provided written consent for CSF spiking

experiments. The CSF obtained was spiked with serial dilutions of *M.tb.* (H37Rv). C_T values were also correlated with bacterial load (MGIT 960 time to culture positivity (TTP), and PCR inhibition was evaluated by comparing the C_T value of the internal positive control (IPC; *Bacillus globigii*) in CSF to that in sputum samples obtained from a previously described reference cohort (8).

Statistical methods

Characteristics of definite and non-TBM patients were compared using Chi Square or Fisher's exact test for categorical variables and Wilcoxon's Rank Sum test for continuous variables. Sensitivity, specificity, positive and negative predictive values, overall agreement and likelihood ratios (LR) are reported as measures of diagnostic efficacy. Specificity and sensitivity comparisons between Amplicor PCR and Xpert® MTB/RIF were done using McNemar's Chi Square test. Data was analysed using Stata v12 (Statacorp, USA).

We calculated the LR as the ratio of the probability of a positive test among the truly positive subjects to the probability of a positive test among the truly negative. The LR- is the ratio of the probability of a negative test among the truly positive subjects to the probability of a negative test among the truly negative subjects.

The actual number calculation in the table 2 of the manuscript is explained below.

Amplicor PCR:

A total of 127 patients had Amplicor results. Of these 46 patients had confirmed TBM and 81 were non-TBM. Of the patients with TBM, 21 patients tested positive on Amplicor (sensitivity = 21/46 = 46%). Of the 81 patients with non TBM, 80 tested negative on Amplicor (specificity =80/81=99%).

Of the 22 patients who were positive on Amplicor, 21 have confirmed TBM (positive predictive value = 21/22 = 95%).

Of the 105 patients who tested negative with Amplicor, 80 patients were non-TBM (negative predictive value = 80/105 = 76%).

For agreement, 21 patients were both positive on Amplicor and had TBM and 80 patients were both negative on Amplicor and were non-TBM, therefore 101 (21+80) had test results consistent with disease status out of 127 patients tested (agreement = 101/127=80%)

Amplicor	**TBM***	**Non-TBM**	**Total**
Positive	21	1	22
Negative	25	80	105
Total	46	81	127

*Using positive liquid culture or microscopy as reference standard for TBM

Xpert® MTB/RIF:

A total of 89 patients had Xpert® MTB/RIF results. Of these 36 patients had confirmed TBM and 53 were non-TBM. Of the patients with TBM, 18 patients tested positive on Xpert® MTB/RIF (sensitivity = 18/36 = 50%).

Of the 53 patients with non-TBM, 50 tested negative on Xpert® MTB/RIF (specificity: 50/53=94%). Of the 21 patients who were positive on Xpert® MTB/RIF, 18 have confirmed TBM (positive predictive value = 18/21 = 86%).

Of the 68 patients who tested negative on Xpert® MTB/RIF, 50 patients were non-TBM (negative predictive value: 50/68 = 74%).

For agreement, 18 patients were both positive on Xpert® MTB/RIF and had TBM and 50 patients were both negative on Xpert® MTB/RIF and were non-TBM, therefore 68 (18+50)

had results consistent with their disease state out of a total of 89 patients tested

(agreement = 68/89=76%).

Xpert® MTB/RIF	TBM*	Non TBM	Total
Pos	18	3	21
Neg	18	50	68
Total	36	53	89

*Using positive liquid culture or microscopy as reference standard for TBM

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